

# RNA Extraction from maize callus and leaf tissues

## Materials

- ⌘ Trizol (Invitrogen cat# 15596-026)
- ⌘ Chloroform: IsoAmyl Alcohol (24:1, v:v)
- ⌘ Isopropanol
- ⌘ DEPC H<sub>2</sub>O
- ⌘ 75% Ethanol (with DEPC treated water)

## Methods

### ⌘ Preparation of DEPC Water

1. Add 0.1% of DEPC (diethylprocarbonate) in water. Mix well by shaking the bottle. (DEPC is highly toxic so every thing should be done under the fume hood with a maximum of protections)
2. Incubate overnight under the fume hood.
3. Loosen up the lid and autoclave 20 min on liquid cycle. DEPC gets degraded (hydrolysis) during the process so autoclaved DEPC water can be considered as non-toxic after completion of a proper autoclave cycle.
4. Store DEPC water at room temperature for several weeks.

### ⌘ Preparation of Utensils for RNA Extraction

1. Soak plastic Kimble pestles used for callus RNA extraction in 30% Hydrogen Peroxide for 15 minutes. Rinse with DEPC water. The Hydrogen Peroxide may be used twice before it is discarded. Be extremely careful when using Hydrogen Peroxide, as it will burn skin.
2. Bake mortars, pestles, and spoons for leaf RNA extraction in a 232°C oven overnight.
3. Make sure to have RNase free 1.5 ml microcentrifuge tubes and RNase free filter tips.

### ⌘ RNA Extraction

1. Collect ~200 mg callus (up to the 0.5 ml mark in a RNase free 1.5 ml microcentrifuge tube)

2. Add 500  $\mu$ l of Trizol and grind samples until thoroughly mixed with Hydrogen Peroxide treated plastic Kimble pestles. For leaf tissue, use baked mortar and pestles and liquid N<sub>2</sub> to grind the samples. Make sure to use RNase free filter tips for everything.
3. Add 500  $\mu$ l more of Trizol and vortex well. Let sit 10 minutes at room temperature.
4. Add 200  $\mu$ l of chloroform: isoamyl alcohol and vortex well. Let the tubes sit 10 minutes at room temperature then spin at 4°C for 15 minutes at maximum speed on a desktop microcentrifuge.
5. Carefully pipette out the top aqueous phase into clean 1.5 ml microcentrifuge tubes. Be careful not to take any of the bottom layer of liquid.
6. Add 500  $\mu$ l of Isopropanol. Leave on bench for 10 minutes. Can also leave at -80°C until you are ready to finish the protocol.
7. Pellet the DNA by centrifuging as in step 4.
8. Decant isopropanol
9. Add 500  $\mu$ l 70% Ethanol (DEPC treated) and re-suspend the pellet with a pipet tip. Centrifuge as in step 4 for at least 5 minutes and decant the isopropanol.
10. Invert tubes on paper towel and allow the pellets to air-dry for 5-15 minutes until no droplets can be seen inside the tube.
11. Re-suspend pellet in ~20  $\mu$ l of RNase free DEPC water (Actual volume will depend upon the size of the pellet).